

REMARKS

Claims 1-3, 5-8 and 12-17 are pending. Claims 11-14 and 18 have been withdrawn from consideration. Claims 1-3, 5-9 and 15-17 have been rejected. Claims 19 and 20, corresponding to canceled claims 11 and 18, respectively, have been added. Claims 1-3, 5-8, 12-17, 19 and 20 remain in the case.

The examiner's stated rejection

A rejection under §103(a) based on Zollinger et al. ("Zollinger") has been withdrawn, and a §103(a) rejection based on Zollinger in combination with Ziegler et al. ("Ziegler"), Myers et al. ("Myers") and Munford et al. ("Munford") has been newly stated. Zollinger discloses the use of capsular polysaccharide or lipopolysaccharide to solubilize an outer membrane protein.

The examiner admits that Zollinger does not teach J5 *E. coli* polysaccharide complexed with meningococcal outer membrane protein, but urges that a skilled artisan would have been motivated to use LPS from the J5 strain instead of LPS from other strains "as an immunogen to treat sepsis caused by multiple gram negative bacterial pathogens." As further support for the substitution, the examiner notes that Myers teaches that "the core region is highly conserved among LPSSs obtained from different genera of *Enterobacteriaceae*" and that *E. coli* J5 has "a partially complete (and therefore antigenically cross-reactive) core region."

The teachings in Myers and Munford, taken in light of the art as a whole, would not have suggested that J5 LPS could form the basis for an effective active vaccine against sepsis

Myers describes a method for preparing detoxified LPS, and does not suggest that such a preparation can be used as an active vaccine to elicit anti-endotoxin antibodies for therapy. Indeed, the present inventors have found that detoxified LPS, given alone or as a conjugate, does not induce broadly cross-reactive antibodies.

Myers' disclosure that the core region is highly conserved, relates a fact long known. While there are highly conserved epitopes in the LPS core, a microheterogeneity now is known to exist in these epitopes. See, e.g., Table 1 in the manuscript "Vaccines and Antibodies in the Prevention and Treatment of Sepsis" and Figure 11 of Lugowski (copies appended). It was not surprising, therefore, that studies by Lugowski et al. (1996), in which core LPS from *E. coli* was used as a vaccine, revealed no binding to *Klebsiella* (applicants' J5 LPS/OMP vaccine does bind to *Klebsiella*, see below). Moreover, there was little cross reaction between antiserum raised against the core LPS of J5 and other cores from *E. coli*, including the prototype core R3 to which J5 *E. coli* belongs! Thus, even within *E. coli*, there are significant differences between core epitopes.

Di Padova (copy appended) similarly suggests a conserved core region by disclosing that a monoclonal antibody binds to the 5 known cores of *E. coli* and to

Salmonella core. Here again, however, there was no binding to *Klebsiella*. There also was no binding to *P. aeruginosa* (applicants' J5 LPS/OMP vaccine also binds to *P. aeruginosa*, see below).

The examiner also cites Munford in support of the obviousness of substituting J5 LPS for LPS that contains O-chains. Munford discloses that "the structure of the Lipid A moiety is highly conserved." The Lipid A moiety is the portion of lipopolysaccharides that is responsible for toxicity (Munford, col. 1, lines 44-45), and is modified in both Munford and in the present invention to avoid toxicity. The fact that the Lipid A moiety is highly conserved has little bearing on the issue of obviousness in the present case. This is so because numerous experimental studies, with polyclonal anti-lipid A antibody, and four studies in humans with anti-lipid A monoclonal all have shown that antibody against this *most conserved of core LPS structures* is *completely without activity in the treatment of sepsis*. Munford himself later wrote an editorial in the *New England Journal of Medicine*, critiquing the failure of anti-lipid A monoclonal antibodies. Accordingly, the disclosure in Munford relating to lipid A would not have suggested the present invention, and also shows that efficacy as a vaccine cannot be predicated on conservation in structure alone.

Finally, the disclosure in Munford that the R core is "similar in most gram-negative bacteria," that also is cited by the examiner, must be balanced against Munford's uncited disclosure that "the O-antigen is the most antigenic component of the LPS" and the fact that Munford

uses LPS with O-chains, modifying only the Lipid A moiety. Accordingly, Munford would not have suggested to a skilled artisan to use the R core in a vaccine formulation.

In short, the art as whole contravenes any argument that there is a single highly-conserved core region, and would not have suggested that J5 LPS could form the basis for an effective active vaccine against sepsis. While Myers and Munford may guide the artisan to ways of making LPS less toxic by modifying the lipid A moiety, they offer no guidance in making an effective therapeutic formulation for active vaccination against sepsis. It is applicants' combination of detoxified J5 LPS and OMP that presents the LPS to the immune system in an efficacious manner.

Further evidence that teachings like those in Myers and Munford relating to a conserved core region and/or conserved lipid A moiety would not have suggested the present invention is found by searching the literature for disclosures of lipopolysaccharide. A recent search by Dr. Cross for disclosures relating to "lipopolysaccharide" in PubMed (the internet access to Medline) resulted in 30,621 documents. Of these, only 739, or 2.4%, dealt with a "lipopolysaccharide vaccine." No disclosures of a J5 LPS/OMP vaccine other than applicants' were found. The appended review article by Cross et al. lists all published studies that used an active vaccine for the purpose of inducing antibodies to protect against sepsis. All of the studies used whole, killed bacterial preparations. None used purified LPS either alone or formulated with another component.

Yet further evidence of unobviousness is found in an NIH review of a grant proposal by Dr. Cross to study J5 LPS/OMP vaccine. Competition for NIH grants is fierce, and proposals are subjected to rigorous reviews by experts in the field. Appended to this response are the comments of the reviewers. The examiner will particularly note the comments on page 5, under the heading "Innovation" that while "the notion of actively or passively immunizing subjects against highly conserved epitopes in the deep core region of the LPS molecule is an old idea...exhaustively explored" that:

it would be novel, and indeed quite exciting, if it were shown to be possible to actively immunize humans against highly conserved LPS antigenic determinants using a potent and yet nontoxic vaccine. *The investigator's preliminary data support just this possibility.* Furthermore, the preliminary data obtained in animals suggest that the anti-J5 antibodies developed in response to the vaccine are protective against *heterologous* Gram-negative infection. Accordingly, the proposal is, in fact, *innovative and clever.* (emphasis added)

The third critique in the NIH grant review, on page 6, states that:

paradoxically, the major innovation may consist in *returning to a battle most have conceded.* Narrowly, the innovation consists of the form in which the antigen is presented. No immunologic theory is adduced to explain why this appears, in preliminary data, to be *so much more effective than other approaches.* However, immunologic theory is not well enough developed for its omission to get in the way of *successful empirical results.* (emphasis added)

The comments of the NIH reviewers are opinions rendered by independent experts in the field of sepsis prevention, regarding the novelty and inventiveness of applicants' claimed vaccine. They are entitled to great weight, therefore, in any assessment of obviousness.

Nothing in Zollinger would have motivated a skilled artisan to use J5 LPS in combination with an outer membrane protein, in place of the LPS disclosed in Zollinger

Zollinger describes a process for preparing detoxified polysaccharide-outer membrane protein complexes. The polysaccharide may be capsular polysaccharide or detoxified lipopolysaccharide, although only capsular polysaccharides are exemplified (complexes with lipopolysaccharides are prepared in Example 3, but never tested for bactericidal antibody response).

The *purpose* of the polysaccharide in Zollinger, whether capsular polysaccharide or lipopolysaccharide, is to solubilize the outer membrane proteins. Thus, Zollinger speaks of "outer membrane proteins...*solubilized* by the tetravalent mixture of A, C, Y, and W135 polysaccharides" (col. 2, lines 7-9), and describes that "the detoxified [lipopolysaccharide] was shown to retain its ability to bind to and *solubilize* outer membrane proteins" (col. 8, lines 66-68); and "sonication is often essential to facilitate the protein-lipopolysaccharide interaction and *solubilize* the protein" (col. 9, lines 13-15), emphasis added in each case. For the purpose of solubilization, either detoxified lipopolysaccharide or capsular polysaccharide can be used.

Since Zollinger does not specifically teach the use of endotoxin derived from J5 mutant, the basis for the present rejection must be that it would have been obvious to substitute endotoxin from this mutant for capsular polysaccharides or for lipopolysaccharide purified from a serogroup B case strain because equivalent results would be achieved, *i.e.*, ***that the J5 LPS would be equally effective in solubilizing the outer membrane protein.*** The premise on which the rejection is based must be that J5 lipopolysaccharide would be expected to behave equivalently in combination with outer membrane protein ***in terms of the ability to solubilize outer membrane protein, since that is the purpose of the LPS in Zollinger.***

Would LPS without O-chains have been expected to solubilize outer membrane protein as effectively as LPS with O-chains? Zollinger did not use LPS without side chains, and so provides no direct guidance on this issue. But Zollinger does comment specifically, at the bottom of column 8, that "***the detoxified product was shown to retain its ability to bind to and solubilize outer membrane proteins***" (emphasis added). That is, Zollinger felt it necessary to comment on whether a modification of LPS to remove part of it, *i.e.*, the Lipid A moiety, would affect its ability to achieve the stated purpose for the LPS in the Zollinger, namely the ability to bind to and solubilize outer membrane protein. This suggests uncertainty over whether an LPS molecule modified to remove Lipid A would retain the necessary solubilizing properties. A skilled artisan might doubt, as well, the ability of LPS without the O-chains effectively to solubilize outer membrane protein, thereby undermining the alleged case of obviousness.

The examiner's argument that a skilled artisan would have been motivated to use LPS from the J5 strain instead of LPS from other strains "as an immunogen to treat sepsis caused by multiple gram negative bacterial pathogens" ignores the fact that Zollinger is not concerned with prevention or treatment of sepsis caused by multiple gram negative bacterial pathogens. The proper viewpoint must be that of the primary reference, i.e., whether the substitution would have been expected to achieve the purpose stated in the primary reference. The purpose of the Zollinger composition is not the prevention or treatment of sepsis, let alone "sepsis caused by multiple gram negative bacterial pathogens," and LPS in Zollinger is not used "as an immunogen."

Zollinger is not concerned with making antibodies that provide broad-based protection against LPS endotoxin-mediated pathology. Zollinger teaches compositions useful for a different purpose, for *serotype-specific* prevention and treatment of infection from homologous, non-Enterobacteriaceal and non-Pseudomonas, strains. The Zollinger complex protects "against infection by *the same bacteria from which it has been derived*" (abstract and column 2, lines 20-29 - emphasis added). Indeed, all seven figures show that the antibodies induced react only with the homologous bacteria from which the vaccine was made. In Example 3, the sole example describing preparation of a complex of outer membrane protein and detoxified LPS, the OMP and LPS are both from serogroup B strains. And Zollinger reports that an OMP-LPS vaccine generates *type-specific antibodies against meningococci that are bactericidal for that one serotype*, noting that

"lower response were seen when the sera were tested against heterologous serotypes."

Thus, Zollinger is not directed to compositions used to prevent or treat septic shock or any other LPS endotoxin-mediated pathology, such as sepsis, as evidenced by their reporting of **only bactericidal responses to the outer membrane protein** portions of polysaccharide-OMP complexes (column 14, lines 37 et seq.). A bactericidal response is one in which antibodies **directly kill bacteria** in the presence of complement. Zollinger does not have as its purpose **protection against LPS endotoxin-mediated pathology**, and therefore Zollinger would not have suggested the possibility of a vaccine to prevent LPS endotoxin-mediated pathology comprising a combination of J5 LPS with OMP. No *prima facie* case of obviousness exists based on the combination of Zollinger in view of Ziegler, Myers and Munford.

The insights underlying applicant's J5 vaccine are entirely different from, and not presaged by, the teachings of, Zollinger. The only similarities to the Zollinger description is the use of the OMP. OMP in Zollinger is used to generate bactericidal antibodies. According to the present invention, on the other hand, the OMP of *N. meningitidis* appears to maintain J5 LPS in a proper spatial configuration such that relevant cross-reactive epitopes in the J5 LPS core are exposed in a manner that they are not when simply conjugated to protein or given alone. A key aspect of applicants' vaccine is reflected in their demonstration that LPS of *E. coli* J5 (Rc chemotype) - the highly conserved core of endotoxin - can produce antibodies that provide protection against the

biologic activities of heterologous LPS, and do not kill bacteria. Thus, antibodies directed against a common epitope in a highly conserved region of LPS interrupts multiple inflammatory mediator cascades initiated by a non-viable portion of the gram-negative bacteria. These antibodies do not prevent infection and applicants' complex does not promote the killing of bacteria, either directly or indirectly.

Even Ziegler's disclosure would not have suggested that the R core of LPS generates antibodies that prevent or treat sepsis

In their landmark study, Ziegler demonstrated that a vaccine made from J5 *E. coli* could induce an antiserum that prevented mortality from septic shock. In that study, however, they were unable to identify antibodies as a basis for that protection. For example, Zanetti et al. (1991), state that "as was noted in the report by Ziegler et al., protection was related to immune plasma, not to specific levels of antibody to core LPS in a given plasma" (copy appended, first paragraph on page 988) and "as already noted, in both successful clinical studies with *E. coli* J5 antiserum, the protection remained of unclear origin because outcome could not be convincingly correlated with the level of antibodies to the core LPS of *E. coli* J5...the protection afforded by *E. coli* J5 antiserum could not be attributable to antibodies to the LPS of *E. coli* J5" (second full paragraph on page 988). Similarly, Glauser et al. (copy appended) note that "a favorable outcome could not be correlated with antibody titers in either of the two clinical studies done with human polyclonal antisera to J5...the mechanisms of protection by antisera to J5 remain unknown" (second full

paragraph on page S208). Baumgartner (copy appended) states that "the successful studies did not discover the factor responsible for the postulated crossprotection in J5 antiserum, because the protection could not be attributed to anti-J5 LPS, anti-Re LPS, or anti-lipid A antibodies" (top of page 923).

Perhaps most telling is the following discussion in Greisman *et al.*, (copy appended):

A particularly puzzling observation was that by Braude and Ziegler, who noted that "equal protection occurs against heterologous LPS with antisera generated against smooth and rough LPS, providing further proof that O antibody is not necessary for preventing death from endotoxin." Since antisera generated against S-form LPS are unreactive with the LPS Rc core of J5 *E. coli*, with the Rd or Re core of *Salmonella*, or with lipid A, this observation strongly implies that factors in J5 and R595 antisera other than antibodies to J5 or R595 LPS core epitopes or lipid A mediate their reputed broad-spectrum protection (page 125, emphasis added).

Thus, even Ziegler herself casts doubts on an assumption that the LPS portion of the J5 boiled bacteria was the active immunogen.

Greisman *et al.* goes on to re-evaluate the effectiveness of broad-spectrum protection by antisera to inner-core LPS epitopes of enterobacteria. Using essential controls, they could not demonstrate broad-spectrum protection with rabbit antisera containing high titers of HA antibodies to Rc (J5) LPS. They also review studies by Ng *et al.*, Peter *et al.*, van Dijk *et al.*, Trautmann and Hahn, Vuipio-Varkila and co-workers,

Baumgartner et al. and Welch et al. all of which "confirmed these negative findings" (page 125, right-hand column). On page 146, they note that the concept that antisera to Rc (J5) do not provide significant broad-spectrum protection is supported by the failure of five clinical trials since Ziegler. They conclude that "controls for [observations of broad-based protection] were often inadequate and that broad-spectrum protection could not be obtained by our laboratory or by many other investigators.

In the nearly fifteen years since the publication of the first J5 clinical study, no investigator has been able to demonstrate a role for J5 antibodies in general and IgG in particular. The teaching of Ziegler herself and the art since Ziegler is that it likely was factors in the antisera other than the LPS that were responsible for the protection observed. Based on this, a skilled artisan would not use a complex of J5 LPS and OMP to generate antibodies effective against LPS endotoxin-mediated pathology, but would have looked for other factors in the Ziegler antisera.

A key feature of applicants' invention is the ability of purified and detoxified J5 LPS antibodies generated in response to a complex of *N. meningitidis* OMP and J5 LPS according to the present invention to mediate protection independently of whole serum. Applicants teach that the IgG isotype that predominates in commercially-available gammaglobulin preparations can provide this protection. As noted above, it is believed that the OMP of *N. meningitidis* appears to maintain J5 LPS in a proper spatial configuration such that relevant cross-reactive

epitopes are exposed in a manner that they are not when simply conjugated to protein or given alone.

Active immunization with applicants' combination of OMP and J5 LPS effectively protects against sepsis

Applicants teach a vaccine that provides effective protection against sepsis. Applicants previously provided evidence in a Rule 132 declaration of Dr. Alan Cross, showing the ability of a vaccination with OMP/J5 LPS vaccine to protect mice from sepsis following a subsequent challenge with a heterologous strain of *E. coli*. The examiner has not commented on the evidence in this declaration.

The examiner's attention again is particularly directed to experiments described in paragraphs 2-5 of this declaration. The experiments examined the efficacy of active immunization with four different vaccines. A first group of mice was immunized only with *N. meningitidis* OMP, a second with *Brucella* LPS complexed to *N. meningitidis* OMP, a third with J5 LPS complexed to OMP, and a fourth with EC018 LPS complexed to OMP. Vaccination with J5-OMP leads to 90% survival, 50% greater protection than vaccination with EC018-OMP, a complex of OMP with another strain of *E. coli*. This is particularly surprising in view of the fact that J5-OMP vaccine was providing protection against a heterologous strain (EC018) whereas EC018-OMP was providing protection against the same strain. The results reported in the Cross declaration could not have been predicted based on Zollinger, which teaches that all capsular polysaccharides and lipopolysaccharides behave equivalently in combination

with an outer membrane protein for the purpose of solubilization as disclosed in Zollinger.

Applicants provide with this response further evidence of the remarkable ability of active immunization with J5 LPS/OMP vaccines to protect against sepsis from heterologous gram-negative strains. A further declaration of Dr. Alan Cross documents studies of challenge with virulent strains of heterologous bacteria following active immunization with J5 LPS/OMP. As described in the protocol appended to the declaration, rats rendered neutropenic with cyclophosphamide were immunized, either with de-O-acylated J5 LPS ("dLPS") complexed to OMP or with saline, in a 3-dose regimen prior to challenge with the heterologous bacteria. Levels of antibody titer for rats immunized with the J5 dLPS/OMP exceeded a target level of 800 ELISA units/ml of antibody, a level previously shown to be protective in passive protection experiments. Following immunization, the rats were challenged with either *Pseudomonas aeruginosa* or *Klebsiella pneumoniae*, in a dose which exceeded LD₉₀ for this experimental model in previous studies.

The results showed that active immunization with J5 dLPS/OMP vaccine produced a prompt and sustained anti-core glycolipid antibody level that was generally in 100-fold excess of pre-immunization baseline levels. Twenty-four hours after bacteremia, antibody levels decreased, but then rapidly recovered to, and remained at, pre-infection levels. Active immunization with J5 LPS/OMP vaccine induced greater than 800 ELISA units/ml of antibody at the onset of neutropenia, nearly 4 weeks after the last dose of vaccine, and this level persisted

throughout the entire period of neutropenia, for up to 80 days after the initial immunization. This is in distinct contrast to results achieved by passive immunization with antibodies, where initial levels of 800 ELISA units/ml of antibody dropped to less than 200 ELISA units/ml of antibody by 24 hours.

Immunization did not prevent either systemic infection or initiation of sepsis, but it clearly reduced the likelihood of a lethal outcome following infections with both heterologous strains of bacteria. Vaccinated animals challenged with *Pseudomonas* had an overall survival rate of 48% compared to 7% for saline treated control animals. A similar result ensued with *Klebsiella* challenge, with a 64% survival rate for vaccinated animals versus a 13% survival rate for control animals.

One particularly surprising result was the effect of the vaccine on organ colonization by the bacteria. Vaccinated animals had the same frequency and magnitude of bacteremia from the challenge strain as the control group, but had significantly lower levels of bacteria in liver and spleen than control animals. Dr. Cross hypothesizes that antibodies generated in response to the vaccine promote the uptake and killing of bacteria from the blood by tissue.

In addition to the decreased bacterial colonization in liver and spleen, there was a significantly lower level of circulating endotoxin at the onset of fever in vaccinated animals as compared to control animals. While endotoxin levels increased in both groups at 24 hours, they were still lower than those of

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the control group. The lower level of circulating endotoxin may be due in part to promotion of LPS clearance from the circulation.

In view of the amendments to the claims and the foregoing remarks, it is believed that all claims are in condition for allowance. Reconsideration of all rejections and a notice of allowance are respectfully requested. Should there be any questions regarding this application, Examiner Devi is invited to contact the undersigned attorney at the phone number listed below.

Respectfully submitted,

January 14, 1999

Date

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